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I, JANENE PEISKER, TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. 2002952492 for a patent by CBIO LIMITED as filed on 06 November 2002.

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JANENE PEISKER
TEAM LEADER EXAMINATION
SUPPORT AND SALES

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Patents Act 1990

PROVISIONAL SPECIFICATION

Invention Title: "CHAPERONIN 10 IMMUNOSUPPRESSION"

The invention is described in the following statement:

TITLE

CHAPERONIN 10 IMMUNOSUPPRESSION

FIELD OF INVENTION

THIS INVENTION relates to a method of treating graft versus host disease and other transplant-related immunological reactions and diseases. More particularly, this invention relates to a method of prophylactic and therapeutic treatment of graft versus host disease using chaperonin 10.

BACKGROUND OF THE INVENTION

Graft versus host disease (GVHD) is a condition that can develop when immunologically-competent cells have been introduced into an individual, for example during bone marrow transplantation. GVHD refers to the immunological process whereby the newly transplanted cells mount a rejection response against host tissue. GVHD can develop after the transplantation or transfusion of bone marrow tissue, haematopoietic stem cells, unirradiated blood products and solid organs containing lymphoid tissue.

There are two types of GVHD, acute and chronic. Acute GVHD develops within the first three months following transplantation and clinical symptoms include dermatitis, enteritis and hepatitis. Chronic GVHD usually develops three months after transplantation and is an autoimmune syndrome affecting multiple organs and tissues.

Donor T cells are responsible for triggering the development of GVHD. Donor T cells recognise the host cell antigens as foreign and respond by proliferating and releasing cytokines which in turn may activate cells of the innate immune system.

Allogeneic bone marrow transplantion or haematopoietic cell transplantation remains the most effective curative therapy for the treatment of hematological malignancies, such as leukaemia, myeloma and lymphoma. Severe acute GVHD is the primary cause of mortality and morbidity during bone marrow transplantation. Chronic GVHD can also result in death and survivors are often severely disabled.

Immunosuppressive drugs play a large part in the prevention, therapeutic treatment and management of acute and chronic GVHD. The drugs may be administered to the patient before and after the transplant. Current drugs used in the therapeutic treatment of GVHD include cyclosporine, methotrexate, tacrolimus, sacrolimus, mycophenolate mofetil and steroids. Immunosuppression regimens often involve the administration of a combination of drugs for maximal effect.

Chaperonin 10 (cpn10) is present in a variety of organisms, from bacteria to humans, and is a member of the heat shock family of proteins (chaperones) which are among the most evolutionary stable proteins in existence. The chaperone molecules are involved in post-translational folding, targeting and assembly of other proteins (Hartman *et al.*, 1992, Proc. Natl. Acad. Sci. USA, 89, 3394-8) but do not themselves form part of the final assembled structure (Ellis et al., 1991, Annu. Rev. Biochem. 60, 321-47). These proteins play essential roles in normal cells but their production is upregulated during cellular stress (eg. metabolic disruption, infection, inflammation, transformation).

It was unexpectedly discovered that chaperonin 10 has the same amino acid sequence as Early Pregnancy Factor (EPF) (Morton *et al.*, International

Publication WO 95/15338). EPF is a pregnancy-associated substance that appears in the maternal serum within 6-24 hr of fertilization (Morton *et al.*, 1974, Nature, 249; 459-460 and Morton *et al.*, 1976, Proc. R. Soc. Lond., 193; 413-9). It is present for at least the first half of pregnancy and is essential for continued embryonic growth and survival (Morton *et al.*, 1987, Current Topics in Developmental Biology 23; 73-92). It is now clear that EPF has many physiological functions and its production is not confined to pregnancy.

It has been reported that EPF can act as an immunosuppressant, release suppressor factors from lymphocytes (Rolfe *et al.*, 1988, Clin. Exp. Immunol. 73, 219-225) and augment the rosette-inhibiting properties of an immunosuppressive anti-lymphocyte serum (Morton *et al.*, 1974 and 1976, supra). EPF can suppress the delayed-type hypersensitivity reaction to trinitrochlorobenzene in mice (Noonan *et al.*, 1979, Nature, 278, 649-51), suppress mitogen-induced lymphocyte proliferation (Athanasas-Platsis, 1993, PhD Thesis, The University of Queensland) and suppress IFN-γ production by CD4+ T cells.

However, there has been no evidence in the prior art as to whether EPF or cpn10 may have potential as an immunosuppressive agent in transplantation, and in particular in the prevention of GVHD. Chaperonin 60, a related heat shock protein, which can also act as an immunosuppressant, has not been shown to possess any therapeutic effects in GVHD. In fact, the prior art teaches that heat shock proteins may have adverse effects on transplantation (Ogita *et al.*, 2000, Transplantation, 69, 2273-2277).

OBJECT OF THE INVENTION

The present inventors have realized the immunosuppressive drugs currently

used for the therapeutic treatment and management of GVHD have the following significant short-comings:

(i) they induce severe side effects, for example, hypertension which may require additional medication for control, nephrotoxicity which occurs in up to 40% of patients and frequently forces the doctor to administer sub-optimal doses of the drug to limit the toxicity, CNS effects such as tremor, headache, depression, paraesthesia, blurred vision and seizures, increased risk of bacterial, fungal or viral infections, increased risk of cancer, particularly skin cancer, loss of appetite, nausea and increased hair growth;

(ii) GVHD is resistant to the drugs in a significant percentage of patients and combination drug therapy is required;

(iii) the drugs are very expensive; and

(iv) the drugs have demonstrated adverse interactions with other therapeutic drugs, such as antibiotics, NSAIDs, anti-epileptics, and antifungals, immunizations, such as rubella and polio, and natural food, such as grapefruit (in the case of cyclosporin).

Therefore there is an enormous demand for the development of a new drug to treat and manage GVHD that has fewer side effects side effects than the treatments currently available and is more efficacious in patients that show a resistance to the current drugs on the market.

The present inventors have unexpectedly discovered that cpn10 possesses enormous clinical potential as a new therapy in the treatment and management of GVHD.

SUMMARY OF INVENTION

In a first aspect, the invention provides a method of treating graft versus host disease (GVHD) including the step of administering to an animal a pharmaceutically-effective amount of chaperonin 10 (cpn10) or a derivative of cpn10.

Preferably, the animal is a mammal.

Preferably, the mammal is a human.

Preferably, the cpn10 protein is Ala1-101 cpn10.

In a second aspect, there is provided a pharmaceutical composition for the therapeutic treatment of GVHD comprising a pharmaceutically-effective amount of cpn10 or a derivative of cpn10, and a pharmaceutically-acceptable carrier, excipient or diluent.

Preferably, the cpn10 protein is Ala1-101 cpn10.

According to this aspect, the invention provides a method of preventing, suppressing or inhibiting the immune response in an animal in response to acute or chronic GVHD and immunological symptoms evoked by cell, tissue or organ transplantation.

According to this aspect, the invention also provides a method of priming the immune system in preparation for cell, tissue or organ transplantation.

In a third aspect, there is provided a pharmaceutical composition for the therapeutic treatment of GVHD comprising a pharmaceutically-effective amount of cpn10 or a derivative of cpn10, at least one immunosuppressive agent and a pharmaceutically-acceptable carrier, excipient or diluent.

Preferably, the cpn10 protein is Ala1-101 cpn10.

Preferably, the immunosuppressive agent is an immunosuppressive drug or a specific antibody directed against B or T lymphocytes or surface receptors that mediate their activation.

Preferably, the immunosuppressive drug is any one of cyclosporin, tacrolimus, sirolimus, mycophenolate mofetil and methotrexate.

In a fourth aspect, there is provided a pharmaceutical composition for the treatment of GVHD comprising a pharmaceutically-effective amount of cpn10, at least one immunosuppressive agent, a steroid and a pharmaceutically-acceptable carrier, excipient or diluent.

Preferably, the cpn10 protein is Ala1-101 cpn10.

Throughout this specification, "comprise", "comprises" and "comprising" are used inclusively rather than exclusively, will be understood to imply the inclusion of a stated integer or group of integers but not the exclusion of any other integer or group of integers.

BRIEF DESCRIPTION OF THE FIGURES

Fig. 1: The amino acid sequence of the modified non-acetylated cpn10 protein referred to as Ala1-101.

Fig. 2: Survival of mice after bone marrow transplantation. Mice were injected subcutaneously with cpn10 (2 μg/dose or 10μg/dose) daily. Control mice were injected with diluent only. * denotes P<0.5, ** denotes P<0.01 versus control.

Fig. 3: The degree of systemic GVHD demonstrated by mice after bone marrow transplantation. Mice were injected subcutaneously with cpn10 (2 μg/dose or 10μg/dose) daily. Control mice were injected

with diluent only. * denotes P<0.5, ** denotes P<0.01 versus control.

DETAILED DESCRIPTION OF INVENTION

The inventors have demonstrated that unexpectedly low doses of cpn10 have significant immunosuppressive activity in an *in vivo* GVHD model and that cpn10 treatment increases the survival rate of mice suffering from GVHD.

This is the first demonstration that cpn10 produces significant immunosuppressive effects and increased survival rate in an *in vivo* allogeneic transplant model. In particular, this is the first demonstration of the beneficial immunosuppressive effects of cpn10 and increased survival rates in an *in vivo* GVHD model. This is also the first demonstration of immunosuppressive effects of a new recombinant form of cpn10 molecule, Ala1-101 cpn10, in an *in vivo* transplant model.

Ala1-101 is a new, recombinant, non-acetylated cpn10 molecule that does not possess the amino acids glycine, serine and methionine (GSM) at the N-terminus, and is expressed in *E coli* in preference to the pGEX GST system of expression (Ryan *et al.*, 1994, FEBS Lett., 337, 152-156).

The present invention provides evidence that surprisingly low doses of cpn10 (Ala1-101) demonstrate significant immunosuppressive effects in an *in vivo* mouse transplantation model.

Mice were administered a bone marrow transplant followed by post transplantation treatments of cpn10 (2 μg/dose or 10 μg/dose daily). The degree of systemic GVHD shown in the mice was assessed by their survival time and by

the clinical symptoms observed, which were recorded and graded according to a clinical scoring system (see Examples section).

Cpn10-treated mice demonstrated a significantly lower clinical score than the control mice indicating that the degree of systemic GVHD was lower in the cpn10-treated mice than the control mice. The amount of cpn10-induced inhibition of GVHD was dose-dependent (Fig. 3).

Survival time in the cpn10-treated animals was significantly higher than the control animals (Fig. 2). At 35 days post bone marrow transplant, 30% of the cpn10-treated animals and 0% of the control animals had survived.

It is noted that the immunosuppressive protection provided by cpn10 was lost as soon as the cpn10 treatment was terminated.

Therefore cpn10 has the potential to become an important therapeutic drug in the treatment of GVHD.

For the purposes of this invention, by "immunosuppressive agent" is meant an agent that can prophylactically or therapeutically suppress an autoimmune or immune response against a transplanted allogeneic or xenogeneic cell, tissue or organ, or to suppress graft versus host disease.

Preferably, the pharmaceutically-effective amount of cpn10 administered to an individual is within the range 0.1-100 mg.

More preferably, the pharmaceutically-effective amount of cpn10 administered to an individual is within the range 0.1-10 mg.

It will be appreciated by the skilled person that the aforementioned pharmaceutically-effective amounts are calculated in terms of a typical 70 kg human. Accordingly, doses may vary depending on the weight, age, sex, general

health and fitness of the individual and any other treatments to which the individual is being subjected. Furthermore, the amount of cpn10 administered will be interdependent with the frequency and timing of administration.

It will also be appreciated that the aforementioned pharmaceutically-effective amounts of cpn10 can be administered to animals, for example, domestic animals and livestock. Doses would vary depending on the weight and type of animal, as would be apparent to those of skill in the art.

The cpn10 administered to a human or other animal may be any form of isolated cpn10, including but not limited to Ala1-101 cpn10, native cpn10, recombinant cpn10-GSM or any derivative protein of cpn10.

Suitable cpn10 nucleotide and amino acid sequences are well known in the art, although for convenience the skilled person is referred to the following mammalian cpn10 sequences:

(i) human cpn10 (NCBI *Entrez* Accession No. U07550; Chen *et al.*, 1994, Biochim. Biophys. Acta, 1219, 189-190)

(ii) mouse cpn10 (NCBI *Entrez* Accession No. U09659; Dickson *et al.*, 1994, J. Biol. Chem., 269, 26858-864); and

rat cpn10 (NCBI *Entrez* Accession No. X71429; Ryan *et al.*, supra).

By "*isolated*" is meant material that has been removed from its natural state or otherwise been subjected to human manipulation. Isolated material may be substantially or essentially free from components that normally accompany it in its natural state, or may be manipulated so as to be in an artificial state together with components that normally accompany it in its natural state. Isolated material may be in native, chemical synthetic or recombinant form.

By "*protein*" is meant an amino acid polymer. The amino acids may be natural or non-natural amino acids D- and L- amino acids, as are well understood in the art.

A "*peptide*" is a protein having no more than fifty (50) amino acids.

A "*polypeptide*" is a protein having more than fifty (50) amino acids.

The term "*nucleic acid*" as used herein designates single-or double-stranded mRNA, RNA, cRNA, RNAi and DNA inclusive of cDNA and genomic DNA.

As used herein, "*derivative*" proteins of the invention are proteins, such as non-acetylated cpn10 Ala1-101 and non-acetylated cpn10-GSM, which have been altered, for example by conjugation or complexing with other chemical moieties or by post-translational modification techniques as would be understood in the art. Such derivatives include amino acid deletions and/or additions to proteins of the invention, or variants thereof.

Other derivatives contemplated by the invention include, but are not limited to, modification to side chains, incorporation of unnatural amino acids and/or their derivatives during peptide, polypeptide or protein synthesis and the use of crosslinkers and other methods which impose conformational constraints on the polypeptides, fragments and variants of the invention. Examples of side chain modifications contemplated by the present invention include modifications of amino groups such as by acylation with acetic anhydride; acylation of amino groups with succinic anhydride and tetrahydrophthalic anhydride; amidination with methylacetimidate; carbamoylation of amino groups with cyanate; pyridoxylation of lysine with pyridoxal-5-phosphate followed by reduction with

$NaBH_4$; reductive alkylation by reaction with an aldehyde followed by reduction with $NaBH_4$; and trinitrobenzylation of amino groups with 2, 4, 6-trinitrobenzene sulphonic acid (TNBS).

The carboxyl group may be modified by carbodimide activation via O-acylisourea formation followed by subsequent derivitization, by way of example, to a corresponding amide.

The guanidine group of arginine residues may be modified by formation of heterocyclic condensation products with reagents such as 2,3-butanedione, phenylglyoxal and glyoxal.

Sulphydryl groups may be modified by methods such as performic acid oxidation to cysteic acid; formation of mercurial derivatives using 4-chloromercuriphenylsulphonic acid, 4-chloromercuribenzoate; 2-chloromercuri-4-nitrophenol, phenylmercury chloride, and other mercurials; formation of a mixed disulphides with other thiol compounds; reaction with maleimide, maleic anhydride or other substituted maleimide; carboxymethylation with iodoacetic acid or iodoacetamide; and carbamoylation with cyanate at alkaline pH.

Tryptophan residues may be modified, for example, by alkylation of the indole ring with 2-hydroxy-5-nitrobenzyl bromide or sulphonyl halides or by oxidation with N-bromosuccinimide.

Tyrosine residues may be modified by nitration with tetranitromethane to form a 3-nitrotyrosine derivative.

The imidazole ring of a histidine residue may be modified by N-carbethoxylation with diethylpyrocarbonate or by alkylation with iodoacetic acid derivatives.

Examples of incorporating unnatural amino acids and derivatives during peptide synthesis include but are not limited to, use of 4-amino butyric acid, 6-aminohexanoic acid, 4-amino-3-hydroxy-5-phenylpentanoic acid, 4-amino-3-hydroxy-6-methylheptanoic acid, t-butylglycine, norleucine, norvaline, phenylglycine, ornithine, sarcosine, 2-thienyl alanine and/or D-isomers of amino acids.

Derivatives may also include fusion partners and epitope tags. Well known examples of fusion partners include, but are not limited to, glutathione-S-transferase (GST), Fc portion of human IgG, maltose binding protein (MBP) and hexahistidine (HIS_6), which are particularly useful for isolation of the fusion protein by affinity chromatography. For the purposes of fusion polypeptide purification by affinity chromatography, relevant matrices for affinity chromatography are glutathione-, amylose-, and nickel- or cobalt-conjugated resins respectively. Many such matrices are available in "kit" form, such as the QIAexpress™ system (Qiagen) useful with (HIS_6) fusion partners and the Pharmacia GST purification system.

In some cases, the fusion partners also have protease cleavage sites, such as for Factor X_a or Thrombin, which allow the relevant protease to partially digest the fusion polypeptide of the invention and thereby liberate the recombinant polypeptide of the invention therefrom. The liberated polypeptide can then be isolated from the fusion partner by subsequent chromatographic separation.

Fusion partners according to the invention also include within their scope "*epitope tags*", which are usually short peptide sequences for which a specific antibody is available. Well known examples of epitope tags for which specific

monoclonal antibodies are readily available include c-myc, haemagglutinin and FLAG tags.

Isolated proteins of the invention (inclusive of fragments, variants, derivatives and homologues) may be prepared by any suitable procedure known to those of skill in the art, including chemical synthesis and recombinant expression.

Preferably, cpn10 is recombinant cpn10.

For example, the recombinant cpn10 protein may be prepared by a procedure including the steps of:

(i) preparing an expression construct which comprises an isolated nucleic acid encoding cpn10, operably-linked to one or more regulatory nucleotide sequences in an expression vector;

(ii) transfecting or transforming a suitable host cell with the expression construct; and

(iii) expressing the recombinant protein in said host cell.

An "*expression vector*" may be either a self-replicating extra-chromosomal vector such as a plasmid, or a vector that integrates into a host genome.

By "*operably-linked*" is meant that said regulatory nucleotide sequence(s) is/are positioned relative to the recombinant nucleic acid of the invention to initiate, regulate or otherwise control transcription.

Regulatory nucleotide sequences will generally be appropriate for the host cell used for expression. Numerous types of appropriate expression vectors and suitable regulatory sequences are known in the art for a variety of host cells.

Typically, said one or more regulatory nucleotide sequences may include, but are not limited to, promoter sequences, leader or signal sequences, ribosomal binding sites, transcriptional start and termination sequences, translational start and termination sequences, splice donor/acceptor sequences and enhancer or activator sequences.

Constitutive or inducible promoters as known in the art are contemplated by the invention and include, for example, tetracycline-repressible and metallothionin-inducible promoters. The promoters may be either naturally occurring promoters, or hybrid promoters that combine elements of more than one promoter.

In a preferred embodiment, the expression vector contains a selectable marker gene to allow the selection of transformed host cells. Selectable marker genes are well known in the art and will vary with the host cell used.

Suitable host cells for expression may be prokaryotic or eukaryotic, such as *Escherichia coli* (DH5α for example), yeast cells, SF9 cells utilized with a baculovirus expression system, CHO cells, COS, CV-1 and 293 cells, without limitation thereto.

The recombinant cpn10 protein may be conveniently prepared by a person skilled in the art using standard protocols as for example described in Sambrook *et al.*, MOLECULAR CLONING. A Laboratory Manual (Cold Spring Harbor Press, 1989), incorporated herein by reference, in particular Sections 16 and 17; CURRENT PROTOCOLS IN MOLECULAR BIOLOGY Eds. Ausubel *et al.*, (John Wiley & Sons, Inc. 1995-1999), incorporated herein by reference, in particular Chapters 10 and 16; and CURRENT PROTOCOLS IN PROTEIN

SCIENCE Eds. Coligan *et al.*, (John Wiley & Sons, Inc. 1995-1999) which is incorporated by reference herein, in particular Chapters 1, 5 and 6.

An example of production and purification of recombinant synthetic cpn10 using the pGEX system is provided in WO 95/15338. A high yielding bacterial expression system known to produce active cpn10 (Ryan *et al.*, supra) was used to produce the cpn10 Ala1-101. Other examples of methods useful for recombinant protein expression can be found in Chapters 5-7 of CURRENT PROTOCOLS IN PROTEIN SCIENCE (Eds. Coligan *et al.*, John Wiley & Sons Inc., 1995-99), which are herein incorporated by reference.

Pharmaceutical compositions

The invention provides a use of cpn10 for the therapeutic treatment of diseases or medical conditions caused by cell, tissue or organ transplantation, in particular GVHD.

The invention also provides pharmaceutical compositions that comprise cpn10.

Suitably, the pharmaceutical composition comprises an appropriate pharmaceutically-acceptable carrier, diluent or excipient.

By "*pharmaceutically-acceptable carrier, diluent or excipient*" is meant a solid or liquid filler, diluent or encapsulating substance that may be safely used in systemic administration. Depending upon the particular route of administration, a variety of carriers, well known in the art may be used. These carriers may be selected from a group including sugars, starches, cellulose and its derivatives, malt, gelatine, talc, calcium sulfate, vegetable oils, synthetic oils, polyols, alginic acid, phosphate buffered solutions, emulsifiers, isotonic saline and salts such as

mineral acid salts including hydrochlorides, bromides and sulfates, organic acids such as acetates, propionates and malonates and pyrogen-free water.

A useful reference describing pharmaceutically acceptable carriers, diluents and excipients is Remington's Pharmaceutical Sciences (Mack Publishing Co. N.J. USA, 1991) which is incorporated herein by reference.

Any safe route of administration may be employed for providing a patient with the composition of the invention. For example, oral, rectal, parenteral, sublingual, buccal, intravenous, intra-articular, intra-muscular, intra-dermal, subcutaneous, inhalational, intraocular, intraperitoneal, intracerebroventricular, transdermal and the like may be employed. Intra-muscular and subcutaneous injection is appropriate, for example, for administration of immunogenic compositions, vaccines and DNA vaccines.

Dosage forms include tablets, dispersions, suspensions, injections, solutions, syrups, troches, capsules, suppositories, aerosols, transdermal patches and the like. These dosage forms may also include injecting or implanting controlled releasing devices designed specifically for this purpose or other forms of implants modified to act additionally in this fashion. Controlled release of the therapeutic agent may be effected by coating the same, for example, with hydrophobic polymers including acrylic resins, waxes, higher aliphatic alcohols, polylactic and polyglycolic acids and certain cellulose derivatives such as hydroxypropylmethyl cellulose. In addition, the controlled release may be effected by using other polymer matrices, liposomes and/or microspheres.

The above compositions may be administered in a manner compatible with the dosage formulation, and in such amount as is pharmaceutically-effective.

The dose administered to a patient, in the context of the present invention, should be sufficient to affect a beneficial response in a patient over an appropriate period of time. The quantity of agent(s) to be administered may depend on the subject to be treated inclusive of the age, sex, weight and general health condition thereof, factors that will depend on the judgement of the practitioner.

So that the present invention may be more readily understood and put into practical effect, the skilled person is referred to the following non-limiting examples.

EXAMPLES

Materials and Methods

Transplantation

Mice were transplanted according to a standard protocol as described in Hill *et al.*, 1997, Blood, 90, 3204-3213, and Hill *et al.*, 1999, J. Clin. Invest., 104, 459-467. On day 0 B6D2F1 mice received 1400 cGy total body irradiation (TBI, ^{137}Cs source) in two doses separated by three hours to minimize gastrointestinal toxicity. 5×10^6 bone marrow cells and 2×10^6 nylon wool purified splenic donor T cells from B6 mice (allogenic) or B6D2F1 mice (syngeneic) were resuspended in 0.25 ml of Leibovitz's L-15 media and injected intravenously into the irradiated recipients.

Cpn10 treatment

Recombinant human cpn10 was diluted in PBS before injection. Mice were injected subcutaneously with cpn10 (Ala1-101) each day (2 μg/dose and 10 μg/dose) from day 0 to 7 after BMT. Mice from the control groups received injection of diluent only.

Assessment of GVHD

The degree of systemic GVHD was assessed by survival and by a scoring system which sums changes in five clinical parameters: weight loss, posture (hunching), activity, fur texture and skin integrity (maximum index = 10). Individual mice were ear-tagged and graded weekly from 0 to 2 for each criterion. Animals with severe clinical GVHD (scores > 6) were sacrificed according to ethical guidelines and the day of death deemed to be the following day.

Statistical analysis

Survival curves were plotted using Kaplan-Meier estimates and compared by log-rank analysis. The Mann Whitney-U Test was used for the statistical analysis of clinical scores. $P<0.05$ was considered statistically different.

Results

The experiment was terminated at day 35 and suggests the following:

(i) cpn10 inhibits graft-versus host disease in a dose-dependent fashion. At Day 35 post bone marrow transplant, the survival rate was 30% in cpn10 treated animals and 0% in control ($P<0.05$ and $P<0.01$ for 2μg/day and 10 μg/day groups respectively) (Fig. 2).

(ii) Cpn10-treated mice demonstrated a significantly lower clinical score than control mice (Fig. 3).

(iii) The protection provided by cpn10, as determined by the clinical score, was lost when cpn10 administration was stopped at Day 7.

Therefore mice treated with cpn10 demonstrated significantly longer survival time after bone marrow transplantation compared to the control mice. The degree of systemic GVHD was significantly lower in the cpn10-treated mice than in the control mice.

The inventors will determine the levels of inflammatory and anti-inflammatory cytokines generated *in vivo* in control and cpn10-treated animals, and the protective phenotype of the T cell generated *in vivo*. Molecular mechanisms of protection will be determined using antagonists and knockout animals.

Throughout the specification the aim has been to describe the preferred embodiments of the invention without limiting the invention to any one embodiment or specific collection of features. It will therefore be appreciated by those of skill in the art that, in light of the instant disclosure, various modifications and changes can be made in the particular embodiments exemplified without departing from the scope of the present invention.

All computer programs, algorithms, patent and scientific literature referred to herein is incorporated herein by reference.

<u>DATED</u> this sixth day of November 2002

CBIO LIMITED

by its Patent Attorneys

FISHER ADAMS KELLY

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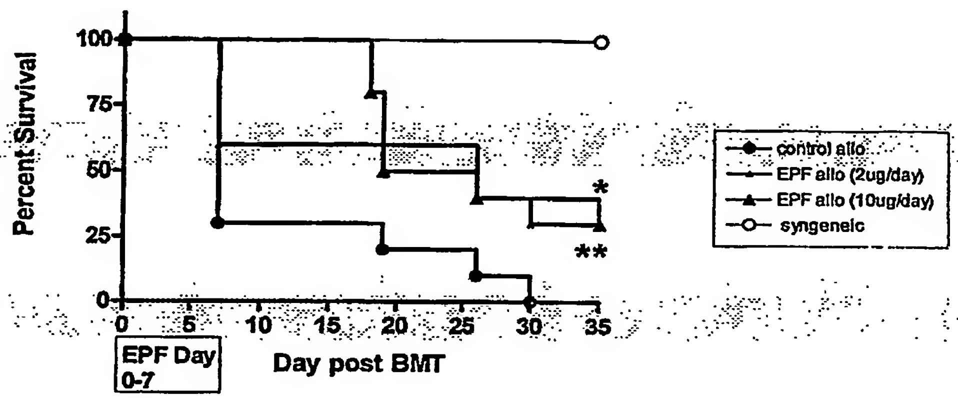
D

FIG. 1


100
75
50
25
0
Percent Survival
0
5
10
15
20
25
30
35
EPF Day 0-7
Day post BMT
*
**
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EPF allo (10ug/day)
syngeneic

FIG. 2

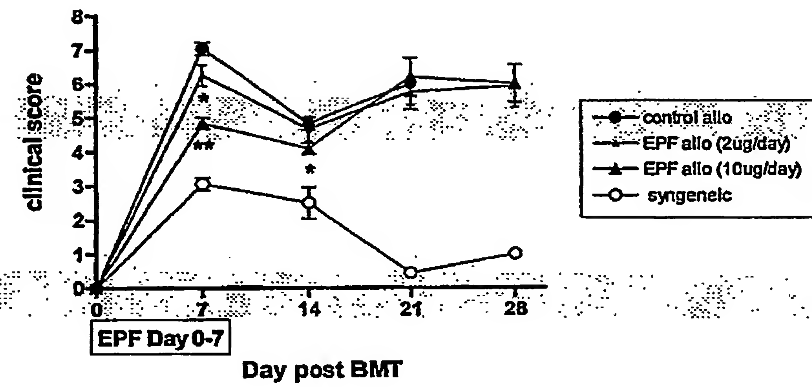
clinical score
0
1
2
3
4
5
6
7
8
0
7
14
21
28
EPF Day 0-7
Day post BMT
control allo
EPF allo (2ug/day)
EPF allo (10ug/day)
syngeneic
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FIG. 3